Aus der Medizinischen Forschungsabteilung der
F. Hoffmann-La Roche & Co. AG., Basel
Arbeit unter Leitung von PD Dr. A. Pletscher

On the Significance of Endogenous 5-Hydroxytryptamine in the Regulation of Water Diuresis

Inaugural-Dissertation
zur Erlangung der Doktorwürde
der Medizinischen Fakultät der Universität Basel

vorgelegt von

BERTRAM SCHNITZER
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5-Hydroxytryptamine (serotonin, 5HT) has been found in various mammalian tissues (1, 2). The greatest amount of the amine is generally present in the gastrointestinal tract (1–4), where it is probably located in the enterochromaffin cells (5). Other tissues which contain 5HT are blood platelets (4–8), brain (9, 10) and mast cells (11).

The metabolic pathways of 5HT in mammals are partly known. Approximately 1% of the primary precursor of the amine, l-tryptophane, is oxidized to 5-hydroxytryptophane and then decarboxylated to 5HT (12–16). Finally, 5HT undergoes oxidative deamination which results in the formation of 5-hydroxyindole acetic acid (5HIAA) (17–19). This acid is normally excreted in the urine of mammals and man (20–21). One of the most important enzymes for the breakdown of 5HT to 5HIAA is monoamine oxidase (22, 17, 18). This enzyme can be found in various tissues, e.g. liver, brain, kidney, intestine, mast cells (1). It seems to be very active since the pharmacological effect of 5HT as well as its biological half life are relatively short (half life of injected 5HT in mice approximately 80 minutes) (23).

The physiological role of 5HT in the body is not yet clear. It has been claimed that the amine plays a role in the regulation of kidney function (1, 20–22, 24), blood pressure (25) as well as in hemostasis (26, 27) and brain function (28). None of these hypotheses, however, has been conclusively proven.

The opinions regarding the role of the amine in the regulation of kidney function are not unanimous. Experiments supporting as well as denying any specific action of 5HT have been reported. "Physiological action" on kidney function has been ascribed to 5HT as a result of experiments carried out on a large number of rats with doses as low as 4 γ of 5HT base per kg body weight applied subcutaneously (1). Other experiments have shown a significant antidiuretic action of 5HT in rats with doses of 10 γ/kg (29) and 0.5 mg/kg (30) body weight respectively. In the dog 0.04–0.8 mg/kg 5HT produced a reduction in urine volume (31).

Contrary to the above positive findings are experiments performed on rats (32), dogs (32, 33) and humans (34) which failed to produce a marked antidiuretic action of 5HT. In rats, doses of 130 γ/kg showed no significant antidiuresis. In dogs, antidiuretic action resulted only when both arterial and venous blood pressure were elevated. The effective dose was 10–20 γ/kg intravenously. Single applications of 5 mg serotonin in humans caused no significant changes in thiosulfate and para-aminohippuric acid (PAH) clearance. Patients with diabetes insipidus receiving 5HT-hydrochloride also showed no change in PAH and inulin clearance (35).

Several theories regarding the possible mechanism of antidiuretic action

of 5HT have been postulated. These include a direct action on the renal vascular system, the tubules or an indirect effect via hypophysis.

Experiments with thiosulfate, creatinine and PAH clearance suggested that antidiuresis in the rat is primarily due to reduction in the glomerular filtration rate (1). According to another theory, the vasa recta as a consequence of afferent vasospasm resulting in divergence of blood to the medulla are concerned with an increase in reabsorption of filtrate (36). Reabsorption of water through direct action on the kidney tubules of the rat has also been ascribed to 5HT (1). Another possible mechanism consists in a primary effect on the posterior pituitary gland which leads to release of antidiuretic hormone (32). Such a mode of action seems, however, unlikely. 5HT caused antidiuresis with a decreased content of chloride in the urine while application of posterior pituitary extract (0.5 U/kg) had an antidiuretic action equal to that of 5HT, but showed the opposite effect on urinary chlorides (30).

These investigations of the role of 5HT in kidney function were carried out following parenteral application of exogenous 5HT. From the results of these experiments, however, no definite conclusions can be drawn concerning the physiological function of endogenous 5HT in diuresis. Thus it is possible that exogenous 5HT hardly penetrates cellular membranes and acts differently than endogenous intracellular 5HT. A different approach to explore the physiology of 5HT consists in using pharmaca which have an effect on the metabolism of endogenous 5HT.

At present there are two known groups of pharmaca which influence the 5HT metabolism of various organs. These are:

1. Rauwolfia alkaloids with tranquillizing effect, e.g. reserpine (37).
2. Substances which inhibit monoamine oxidase, e.g. isopropyl isonicotinic acid hydrazide (iproniazid, Marsilid[1]).

Reserpine causes a decrease of the 5HT content in various body depots (intestine, blood platelets, brain etc.) (38–43). This results in an increase of the excretion of 5HIAA, the major metabolic end product of 5HT, in the urine (43). The greatest part of this increased output of 5HIAA is probably derived from the gastrointestinal tract, the major body depot of 5HT (38). The mechanism of action by which reserpine influences the metabolism of 5HT is not definitely known. It is possible that the drug causes a release of stored or bound 5HT from the tissue; subsequently the amine is probably degradated by monoamine oxidase and then excreted in the urine as 5HIAA (37, 38, 44).

Iproniazid is a potent inhibitor of monoamine oxidase in vitro and in vivo (45–48). Injection of this drug in animals results in a long lasting increase of 5HT in brain (49–51) and blood platelets (52). Guinea pigs show a rise of intestinal 5HT following iproniazid administration which is, however, absent in the rat (53, 54). Furthermore, the biochemical effect of reserpine is antagonized by iproniazid (53, 54). Thus iproniazid counteracts the reserpine induced decrease of 5HT in brain and to a somewhat lesser extent in

[1] Trade name.

the intestine (55). In rats it is not known whether iproniazid inhibits the reserpine induced 5HIAA excretion in the urine as well as the normal 5HIAA excretion.

The above mentioned iproniazid induced changes of the 5HT metabolism may be partly explained by the inhibition of monoamine oxidase. This subsequently leads to an inhibition of 5HT breakdown and to an accumulation of the monoamines in the tissue (54). The antagonistic action of iproniazid concerning the reserpine induced 5HT decrease in brain and intestine is difficult to explain. From histochemical experiments with the enterochromaffin cells it seems possible that iproniazid inhibits the reserpine induced liberation of 5HT from the enterochromaffin cells in the intestine (55).

Reserpine is known to have an antidiuretic effect in rats which have been injected water or saline (24). If endogenous 5HT plays a role at all in kidney function, it may be assumed that this effect is due to the above mentioned action of reserpine on 5HT metabolism. Furthermore, it is conceivable that iproniazid which also alters the 5HT metabolism has an effect on diuresis. In addition, the possibility exists that the antidiuretic effect of reserpine will be diminished by pretreating the animals with iproniazid.

In the present investigation the influence of iproniazid on normal and reserpine induced 5HIAA excretion, as well as on normal diuresis and reserpine induced inhibition of diuresis was studied. By this method it was hoped to gain more information on the role of endogenous 5HT in kidney function. In particular the effect of iproniazid on the following functions was studied in rats:

a) normal 5HIAA excretion,
b) normal water diuresis,
c) reserpine induced 5HIAA excretion,
d) reserpine induced inhibition of water diuresis.

Methods

Measurements of 5HIAA excretion. The urine from groups of four male rats each, weighing 60–90 g, was collected. Following a control period of 24 hours half of the animals received 80 mg/kg iproniazid subcutaneously and 24 hours later all the animals were injected 3 mg/kg reserpine intraperitoneally. Other rats were administered a single dose of 100 mg/kg iproniazid or 50 mg/kg iproniazid daily for three days by subcutaneous route. The 5HIAA content of the urine was colorimetrically (15) determined. The animals received standard laboratory feed and water ad libitum throughout the experiment.

Measurements of diuresis. Diuresis of male rats weighing 60–90 g was measured according to the method of *Burn* (56) following a 16 hour fasting period. The animals were placed in individual metabolic cages and given 5 ml of water by stomach tube. The urine was then collected during a four

hour period following the water application. The diuresis of animals receiving iproniazid and reserpine alone or in combination prior to the water loading were compared to those of the controls which did not receive any drugs. Diuresis was related to the body surface according to the method of *Lee* (57).

Results

1. The application of a single dose of 100 mg/kg of iproniazid or injection of 50 mg/kg iproniazid on three successive days failed to show any change in the 5HIAA excretion in the 24 hour urine (Figure 1, table 1).

2. The 5HIAA content of the 24 hour urine after injection of 3 mg/kg reserpine showed on the average a 100% increase as compared to that without reserpine treatment. This increase was significant ($p < 0.01$). (Table 1).

3. The increase of 5HIAA excretion in the urine caused by reserpine was significantly reduced after pretreatment with 100 mg/kg iproniazid (Table 1).

4. Diuresis was not significantly changed after a single application of 100 mg/kg iproniazid 16 hours before the start of the experiment. Daily

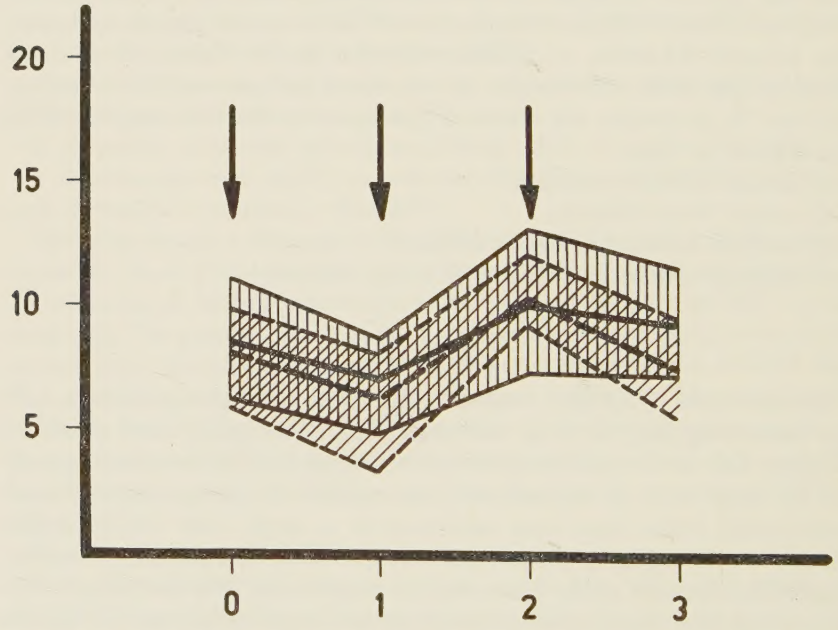

Fig. 1. Influence of iproniazid on urinary 5-hydroxyindole acetic acid (5HIAA) excretion.
Ordinate: 5HIAA excretion in γ per 24 hours per animal. Abscissa: days.
↓ Subcutaneous administration of 50 mg/kg iproniazid; —— extreme values and average of 4 groups of 5 rats each without iproniazid treatment; ---- the same with iproniazid treatment.

Table 1

5-Hydroxyindole acetic acid (5HIAA) excretion in γ per 100 g rat per 24 hours

Before reserpine	Pretreatment with iproniazid after 3 mg per kg reserpine i. p.	Increase after reserpine
13.0*	24.8	+11.8
24.2	24.9	+ 0.7
22.9	34.3	+11.4
21.3	27.9	+ 6.6
18.0	18.7	+ 0.7
10.7	21.5	+10.8
9.7	22.1	+12.4
11.7	19.2	+ 7.5
15.3	21.7	+ 6.4
16.9	24.4	+ 7.5
M= 16.4±1.6 (I)	23.9±1.4 (III)	+ 7.5±1.3 (V)
	Without iproniazid pretreatment	
12.4	38.5	+26.3
16.2	31.1	+14.9
20.6	37.0	+16.4
15.9	34.8	+18.9
19.8	29.9	+10.1
19.5	36.2	+16.7
15.7	22.1	+ 6.4
16.7	35.5	+18.6
19.7	46.1	+26.4
16.1	37.6	+21.5
17.2±0.8 (II)	34.9±2.0 (IV)	+17.7±2.0 (VI)

Significance: I/II $p>0.05$

I/III, II/IV, III/IV, V/VI $p<0.01$

* Each figure represents the result of one group of 4 rats.

injections of 50 mg/kg iproniazid over a period of three days also failed to have an effect on diuresis (Table 2).

5. Reserpine in doses of 2.5 and 8 mg/kg caused a decrease of diuresis to 55% and 44% respectively as compared to that of the controls. Pretreatment with iproniazid 16 hours previous to injection of reserpine did not cause a significant change in the antidiuretic action of reserpine ($p > 0.05$) (Table 2).

6. A dose of 1.5 to 2 mg/kg 5HT injected subcutaneously caused a 50%

Table 2

Influence of iproniazid on diuresis and diuresis inhibition of reserpine

Reserpine dose mg/kg	Without iproniazid pretreatment		Pretreatment with iproniazid		Significance
	No. of animals	Urine volume ml/surface (% of control)	No. of animals	Urine volume ml/surface (% of control)	
0	25	100±18*	25	96±9.4	$p>0.05$
2.5	14	55± 4.4	14	65±8.2	$p>0.05$
8.0	14	44± 3.9	14	41±5.5	$p>0.05$

* Standard deviation

inhibition of diuresis (ED50) in rats, if administered 30 minutes before application of 5 ml of water by stomach tube.

Discussion

Reserpine interferes markedly with the 5HT metabolism in rats. This is shown by the fact that after administration of the drug the 5HIAA output in the urine increases considerably. Similar results in other animal species (dog, rabbit) (37) are confirmed by this finding. It cannot be determined where the 5HIAA which is excreted by the action of reserpine originates. The major part of the acid is probably derived from the 5HT in the intestine, the largest body depot of 5HT, or from the mast cells. The latter have a relatively large content of 5HT in the rat.

Administration of iproniazid for a few days does not seem to alter the 5HT metabolism of the major body depots in rats. This can be concluded by the fact that the drug did not change the 5HIAA excretion in the urine which reflects the metabolic breakdown of 5HT from all the body depots. Furthermore, in earlier experiments, no influence of a single large dose of iproniazid on the 5HT content of the intestine, the largest body depot of 5HT could be observed. On the other hand, iproniazid influences the 5HT metabolism in the brain where it increases the content of the monoamine (53, 54). This change in the 5HT metabolism in the brain is, however, according to our findings, not reflected in a change of 5HIAA excretion. The 5HIAA in the urine originates probably only to a very small extent from the 5HT in the brain, so that changes in the metabolism of brain 5HT do not cause a measurable alteration of the 5HIAA excretion in the urine.

Iproniazid, however, antagonizes the reserpine induced alteration of the 5HT metabolism in the major body depots. This can be concluded from the finding that the reserpine induced excretion of 5HIAA is diminished by pretreatment of the animals with iproniazid. The mechanism of action of this iproniazid effect is not definitely clear. Our finding would be consistent

with the theory that iproniazid inhibits the reserpine induced mobilization of 5HT stored or bound in tissues (see page 5).

If reserpine exerts its antidiuretic effect through a change of the 5HT metabolism (e.g. mobilization of endogenous 5HT) a diminution of the reserpine induced antidiuretic effect by iproniazid should be expected. This is, however, not the case (table 2). Therefore, it can be concluded that the antidiuretic effect of reserpine in rats is probably not due to the influence of the drug on the metabolism of endogenous 5HT.

This view is also supported by considerations on a quantitative basis. In our experiments the increased excretion of 5HIAA in the 24 hour urine following injection of 3 mg/kg reserpine was about 180 γ/kg body weight. This corresponds to approximately 200-250 γ/kg mobilized endogenous 5HT (it is probable that not all the mobilized 5HT can be found as 5HIAA in the urine). The amount of 5HT liberated during the four hours of our diuresis experiments with reserpine was probably less. Thereby the diuresis was inhibited to approximately 50%. The ED50 of 5HT concerning its antidiuretic effect was 1500–2000 γ/kg body weight in our experiments. This dose was considerably higher than the amount of endogenous 5HT mobilized after 3 mg/kg of reserpine.

From these considerations as well as from the results of the experiments with iproniazid-reserpine combination it seems unlikely that endogenous 5HT plays a major role in the regulation of water diuresis in rats.

Summary

The action of the monoamine oxidase inhibitor isopropyl isonicotinic acid hydrazide (iproniazid, Marsilid) on normal and reserpine induced 5-hydroxyindole acetic acid (5HIAA) excretion in the urine as well as on normal diuresis and reserpine induced antidiuresis was investigated in rats. The following results were obtained:

1. Iproniazid had no influence on 5HIAA excretion or water diuresis,

2. The reserpine induced increase of urinary excretion of 5HIAA was significantly decreased by pretreatment of the animals with iproniazid,

3. The reserpine induced antidiuresis was not significantly altered by iproniazid pretreatment,

4. The dose of 5-hydroxytryptamine (5HT, serotonin) which caused 50% diuresis inhibition (ED50) was considerably higher than the quantity of endogenous 5HT which was mobilized by the ED50 of reserpine.

From these experiments the following conclusions can be drawn:

a) The inhibition of the reserpine induced excretion of 5HIAA by iproniazid confirms the theory that iproniazid inhibits the reserpine induced 5HT release from the tissues.

b) The antidiuretic effect of reserpine is probably not the result of the action of the drug on 5HT metabolism.

c) It seems improbable that endogenous 5HT plays an essential role in water diuresis of rats.

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